

# 6

## **APPLICATION OF COATED HOLLOW FIBER PROTECTED MEMBRANE LIQUID PHASE MICROEXTRACTION TO THE ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES**

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This paper evaluates a method for determining the organophosphorus pesticides (methidation, quinalphos and profenofos) in aqueous sample using coated hollow fiber protected liquid phase microextraction system (CHF-LPME) coupled with reversed phase high performance liquid chromatography (RP-HPLC) with UV-Vis detection. Polypropylene fiber coated with polydimethylsiloxane (PDMS) was developed using the sol-gel technology. The physical characteristics of the coated fiber was examined by Fourier transform infrared (FTIR) and field emission-scanning electron microscopy (FE-SEM) equipped with energy dispersive x-ray (EDX). In the preconcentration step, the acceptor phase (4  $\mu\text{L}$ ) introduced into the hollow fiber (1.5 cm) was used to extract organophosphorus pesticides from aqueous sample (11 mL). The limit of detection (LOD) obtained varied from 16.9 to 0.65  $\mu\text{g L}^{-1}$ . The proposed method provided good enrichment factors of up

to 219, with reproducibility ranging from 0.39 to 2.92%, and recoveries of over 75%. This method was applied successfully to the determination of organophosphorus pesticides in selected drinking water samples.

## **6.1 OVERVIEW**

There has been unprecedented growth in measurement techniques over the last few decades. Instrumentations, such as chromatography, spectroscopy and microscopy, as well as sensors and micro devices, have undergone phenomenal developments. Despite the sophisticated arsenal of analytical tools, complete noninvasive measurements are still not possible in most cases. More often than not, one or more pretreatment steps are necessary. These are referred to as sample preparation, whose goals are enrichment, clean up, and signal enhancement.

Sample preparation is often the bottleneck in a measurement process, as they tend to be slow and labor-intensive. However, the past two decades have seen rapid evolution and an explosive growth of this industry. This was particularly driven by the needs of the environmental and pharmaceutical industries, which analyze large number of samples requiring significant effort in sample preparation.

Substantial research has been conducted to miniaturized liquid-liquid extraction (LLE) as sample preparation into liquid microextraction for more than one decade [1,2]. The principle objectives of this have been to reduce the consumption of hazardous organic solvents and to facilitate efficient analyte enrichment without evaporating solvent. There are many improved method, such as solid phase extraction (SPE) [3], solid phase microextraction (SPME) [4], and liquid phase microextraction (LPME) [5].

In SPE, the analytes are trapped by letting the liquid sample flow through the SPE sorbent. The trap is then flushed and the analytes are eluted from the trap with organic solvent, water or a

buffer solution. SPE is still requires an appreciable amount of toxic solvent for analyte desorption [6,7]. Solid Phase Microextraction (SPME) is a solvent free sample preparation method. The technique is simple, fast, and easily operated while it eliminates the advantages of conventional extraction methods, such as time consumption and analyte loss. But SPME also suffers from some drawback. Its fiber is expensive and fragile and has limited lifetime, and sample carry-over is also a problem [8].

More recently, efforts have been placed on miniaturizing the LLE extraction procedure by greatly reducing the solvent to aqueous-phase ratio, leading to the development of liquid phase microextraction (LPME) methodology. LPME provides analyte extraction in a few microliters of organic solvents. It is inexpensive and there is considerable freedom in selecting appropriate solvents for extraction of different analytes. In addition, LPME can be combined with high performance liquid chromatography (HPLC) for the determination of the analytes.

There have been several miniaturizing of LLE in analytical chemistry. The main ideas behind this were to facilitate automation and effectively to reduce the consumption of organic solvents. Miniaturized LLE, or LPME, was introduced in 1996, and involved the used of a droplet of organic solvent hanging at the end of a micro-syringe needle [9-12]. Pedersen-Bjergaard and Rasmussen recently introduced an alternative concept for LPME based on the use of single, low-cost, disposable, porous hollow fibers made of polypropylene (HF-LPME) [13].

The polypropylene fiber commonly used by many researchers is the original fiber without creation. The polypropylene fibers can be modified through coating process using sol-gel technology to increase the functions of mediator to transfer the analytes from donor phase to acceptor phase. Coated fiber is also used to minimize a sample pretreatment from the original sample before analyzed. This case occurred since the pore of the fiber is more selective.

In this research, the polypropylene fiber has been coated by polydimethylsiloxane (PDMS) by sol-gel technology and applied in LPME process to determination of organophosphorus pesticides (methidation, quinalphos, and profenofos) in selected drinking water.

## **6.2 EXPERIMENTAL RESULTS**

### **6.2.1 CHEMICALS AND REAGENTS**

Organophosphorus pesticides (methidation, quinalphos, profenofos and procymidone (as internal standard)) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). HPLC grade acetonitrile and toluene were obtained from J.T Baker (USA). Nonane, ferro sulphate and sodium chloride were obtained from Fluka (Switzerland). Hexane and isooctane were purchased from Merck (Germany). Hydrogen peroxide was obtained from QReC (Germany). Stock solutions of 1000 mg/L of each OPPs were prepared in acetonitrile. Working solutions were prepared by diluting the stock solutions with acetonitrile. The stock solution and working standard were stored in the freezer at about -18°C. Double-distilled deionized water of at least 18 MΩ was purified by Nano ultra pure water system (Barnstead, USA).

The sol-gel substances, methyltrimethoxysilane (MTMOS), hydroxyl-terminated polydimethyl-siloxane (OH-TPDMS), trimethylmethoxy-silane (TMMS), poly(methyl-hydroxysilane) (PMHS) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (USA).

### **6.2.2 HPLC SYSTEM**

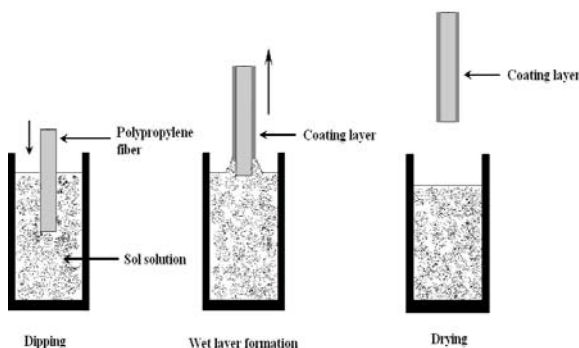
The HPLC system used was a Perkin Elmer Series 200 (USA) that consisted of pump, vacuum degasser, and a UV-Vis detector. The column used (250 mm × 4.6 mm I.D.) was nucleosil

100-5 C18, 5  $\mu\text{m}$  particle size. Acetonitrile-water 60:40 (v/v) mixture was used as the mobile phase at a flow rate  $1\text{ mL min}^{-1}$ . Samples were injected onto the HPLC-UV instrument in triplicates and detected at 200 nm.

### **6.2.3 PREPARATION OF POLYPROPYLENE FIBER AND SOL-GEL SOLUTION**

The polypropylene used in the extraction process was cut manually (1.5 cm length) and sealed at one edge by a sealer machine, then washed with acetone for several min to remove any contaminants. The fiber was then allowed to completely dry and put into a small vial. Fenton's reaction was used to activate the surface of the fiber. The Fenton's reaction was performed by mixing  $\text{FeSO}_4$  and  $\text{H}_2\text{O}_2$  step by step in the vial containing the fiber. After Fenton's reaction was completed the vial containing the fiber was illuminated with UV-ray for 1 h at 365 nm. Then the fiber was dried at the room temperature and ready to be coated.

The polydimethylsiloxane (PDMS) sol phase was prepared by mixing 300  $\mu\text{L}$  MTMOS, 400  $\mu\text{L}$  OH-TPDMS, 20  $\mu\text{L}$  PMHS and 200  $\mu\text{L}$  95 % TFA in a plastic tube. These entire materials were vortexed for 5 min before the mixture was centrifuged at 12,000 rpm for 10 min to produce two layers; the top sol solution was used for fiber coating. The fiber was dipped vertically into the sol solution for 2 min. The coating technique is illustrated in Fig. 1. After the coating process was completed, the coated fibers were then end-capped in a 20% (v/v) methanolic solution of trimethylmethoxysilane (TMMS) for 1 min and then dried at room temperature for 1 day. The resulting coated fiber was submitted for characterization by FE-SEM equipped with EDX analysis. The sol solution was also characterized by FTIR.



**Figure 6.1.** Sol-gel coating technique of PDMS fiber.

#### 6.2.24 LIQUID-PHASE PROCEDURE

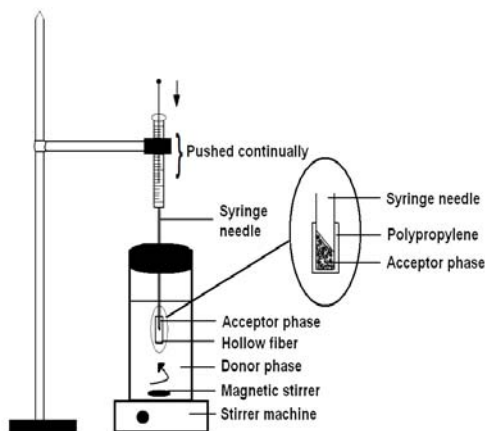
#### MICROEXTRACTION

A 10  $\mu\text{L}$  microsyringe with a cone tip was used to introduce the acceptor phase into the hollow fiber membrane polypropylene. A new hollow fiber was used for each extraction to prevent the carry over effect. The polypropylene fiber was dipped in acceptor phase (e.g. toluene) for 10 seconds to impregnate pores. Microsyringe containing acceptor phase (for example, 3  $\mu\text{L}$  of toluene) was inserted through the septum of the sample vial. The microsyringe needle tip was then inserted into the hollow fiber segment and the assembly was immersed in 11 mL sample solution containing a magnetic stirrer. The plunger pushed slowly to dispense the acceptor phase from the microsyringe into the hollow fiber. The magnetic stirrer was switched on to start the extraction. After extraction for the prescribed time (e.g. 10 min), the plunger of the microsyringe was withdrawn and the acceptor phase obtained was transferred into a small vial (2  $\mu\text{L}$ ) for the drying step at room temperature. After the acceptor phase was completely evaporated, a 2.5  $\mu\text{L}$  of internal standard (procymidone) solution and 47.5  $\mu\text{L}$  of acetonitrile were added into the vial for reconstitution process. A 0.1  $\mu\text{L}$  of the resulting solution was injected into the HPLC for analysis. The LPME set-up is shown in the Figure 6.2.

## 6.3 ANALYSIS AND DISCUSSION

### 6.3.1 CHARACTERIZATIONS OF COATED FIBER

The coated fiber was characterized using Fourier transform infrared spectroscopy (FTIR) and field emission-scanning electron microscopy (FE-SEM) coupled with energy dispersive x-ray (EDX) analyzer. These instrumental methods were used to study the functional group and morphology of the coating.

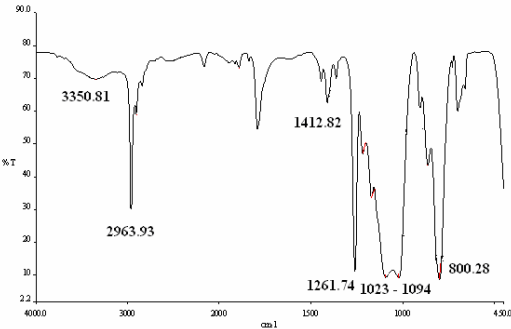


**Figure 6.2.** Schematic of LPME set-up.

### 6.3.2 FOURIER TRANSFORM INFRARED (FTIR)

FTIR was used to identify types of chemical bonds in a molecule by producing an infrared absorption spectrum. The spectrum of sol-gel solution is shown in Figure 6.3. The absorption band at  $3350\text{ cm}^{-1}$  indicates the presence of O-H bonds suggesting incomplete elimination of the hydroxyl group from the end-capping treatment of the activated fiber. The absorption band at  $2963\text{ cm}^{-1}$  shows the stretching of C-H bonds. The sharp absorption

band at  $1261\text{ cm}^{-1}$  and  $800\text{ cm}^{-1}$  and also small absorption band at  $1412\text{ cm}^{-1}$  show the Si bonding with methyl from the sol-gel solution. The broad absorption band around  $1023\text{ cm}^{-1}$  and  $1094\text{ cm}^{-1}$  correspond to stretching of Si-O bonds from Si-O-Si bonds in the sol-gel solution.



**Figure 6.3.** The IR spectra of sol-gel solution.

**Table 6.1.** Infrared frequency ( $\text{cm}^{-1}$ ) for sol-gel PDMS

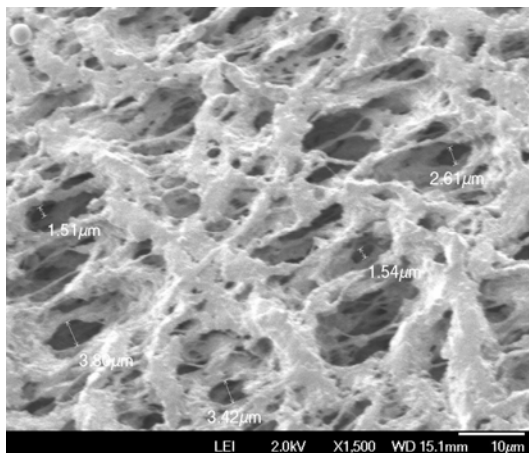
<i>Characteristic</i>	<i>Wave Number (<math>\text{cm}^{-1}</math>)</i>
<i>Vibration</i>	<i>Sol-gel PDMS</i>
O-H	3350 broad
C-H	2963 sharp
Si-C	800, 1261 & 1412
Si-O	1023 & 1094 broad

**6.3.3 FIELD EMISSION-SCANNING ELECTRON MICROSCOPY (FE-SEM) EQUIPPED WITH ENERGY DISPERSIVE X-RAY (EDX)**

According to Stuart [14], an image of the surface of a polymer can be produced by using scanning electron microscopy (SEM). It is used to identify the surface morphology and pore of



the coated fiber. The FE-SEM result is shown in Figure 6.4. The pore size of fiber was obtained for approximately 2.0  $\mu\text{m}$ .

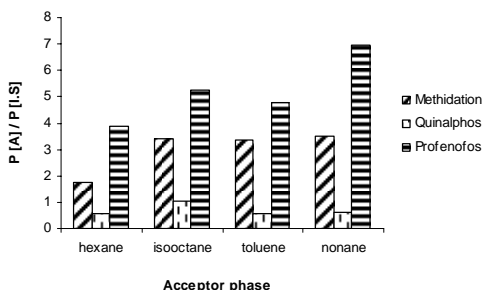


**Figure 6.4.** The SEM image of coated polypropylene.

#### **6.3.4 APPLICATIONS**

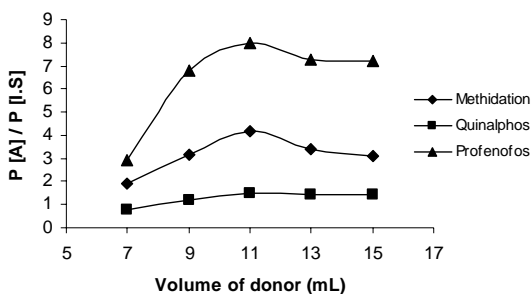
LPME was carried out on various groups of analytes using the coated fiber prepared in this work. The optimization of some parameters (such as acceptor phase, volume of donor and acceptor phase, stirring rate, time of extraction and salting out effect) were studied. A successful extraction is dependant on the acceptor phase. Basically, acceptor phase must have good affinity for target compounds, have low solubility in water so as prevent dissolution into the aqueous phase, and have low volatility which will restrict solvent evaporation during extraction [15,16]. On the basis of these considerations and also considering the polarity of analytes, the use of hexane, isooctane, toluene and nonane were examined. Nonane was found to give the highest analyte recovery (Figure 6.5) and it

was therefore used for subsequent extractions. Nonane is relatively more nonpolar than the other acceptor phases tested and have a high boiling point to avoid the evaporation of acceptor phase during extraction.

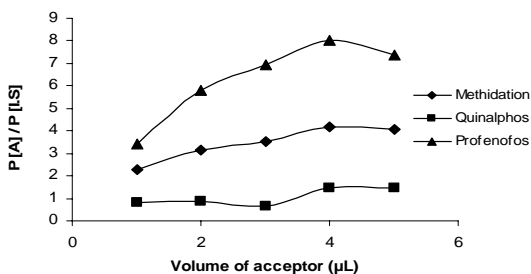


**Figure 6.5.** Acceptor solvent effect of each pesticide.

Volumes of the donor and acceptor phases should be selected by taking into account several considerations. The combination of small acceptor phase volume and large donor phase volume will result in a high enrichment factor of the extraction. In this work, 1 to 5  $\mu\text{L}$  of acceptor phase and various donor phase volumes (7, 9, 11, 13 and 15 mL) were studied. The peak areas of the analytes were found to vary with volumes of donor phase (Figure 6.6) and acceptor phase (Figure 6.7). From the results, 4  $\mu\text{L}$  of acceptor phase and 11  $\mu\text{L}$  of donor phase were found to give optimum results and thus were used in subsequent experiments.

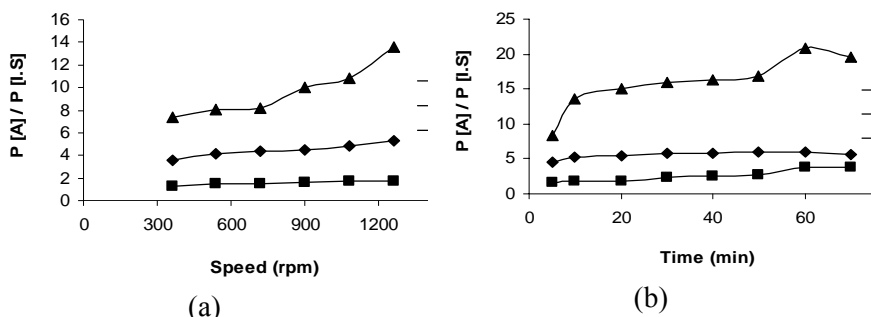


**Figure 6.6.** Volumes variation of donor phases in the extraction



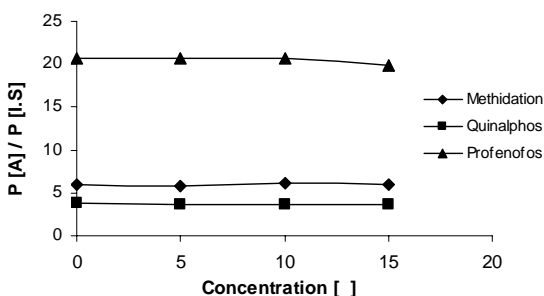
**Figure 6.7.** Volumes variation of acceptor phases in the extraction.

The stirring rate and extraction time play very important role in extraction. Figure 6.8 shows that the relative response increased gradually with increasing stirring rate (360 to 1260 rpm) and time of extraction (5 to 70 min). 60 min and 1260 rpm were found to be the optimum condition. This was probably because faster stirring speed and longer exposure time allow increased rate of mass transfer of the analytes from the donor phase to the acceptor phase to reach equilibrium.



**Figure 6.8.** Effect of (a) stirring speed and (b) extraction time for each pesticide.

It is usual that the addition of salt (NaCl) to the sample can decrease the solubility of analytes in the aqueous phase and enhance their partitioning into the acceptor phase. In this work, the effect of salt was investigated by adding sodium chloride (NaCl) into the aqueous samples for 0, 5, 10, 15 and 25 % (w/v) (Figure 6.9). It was found that the salt affected the polar analyte (methidation) and did not affect the more nonpolar analytes (quinalphos and profenofos). For the best extraction results, therefore, no salt was added to the aqueous sample solution.



**Figure 6.9.** Effect of salting out for each pesticide.

## 6.4 VALIDATION METHOD

The optimum parameters selected were as follows: nonane as acceptor phase, 4  $\mu\text{L}$  for volume of acceptor phase, 11 mL for volume of donor phase, stirring rate of 1260 rpm, 60 min for extraction time and no addition of NaCl in the aqueous solution. The recovery, enrichment factor, limit of detection, linear dynamic range and RSD are listed in Table 6.2.

### 6.4.1 REAL SAMPLE ANALYSIS

The developed extraction method was applied to the analysis of selected drinking water (mineral water) samples obtained commercially from Malaysia market. Because the target compounds were not detected in the samples, each compound was therefore spiked with standard analytes. The results of the analyses are listed in Table 6.3. The recoveries resulted from samples were excellent with values greater than 52.66%. The results suggest that the proposed method can be an effective sample preparation method for the determination of organophosphorus pesticides in drinking water sample matrices.

**Table 6.2.** Analytical performance of CHF-LPME.

<i>Analyte</i>	<i>Recovery (%)</i>	<i>Enrichment factor</i>	<i>Linearity range (<math>\mu\text{g/L}</math>)</i>	<i>Correlation (r)</i>	<i>LODs (<math>\mu\text{g/L}</math>)</i>	<i>RSD (%)</i>
Methidation	84.93	186.84	50 – 750	0.9997	16.9	1.73
Quinlaphos	92.35	203.17	5 – 75	0.9996	1.96	1.41
Profenofos	97.85	215.28	1 - 50	0.9999	0.64	0.52

**Table 6.3.** Recoveries of spiked samples by CHF-LPME

Analytes	Recoveries of real samples (%)		
	Sample A	Sample B	Sample C
Methidation	76.50	71.97	71.51
Quinalphos	79.15	73.74	73.34
Profenofos	82.59	75.11	75.42

## 6.5 CONCLUSION

CHF-LPME technique coupled HPLC-UV provides powerful and efficient method for the analysis of organophosphorus pesticides in water samples. High extraction efficiency was achieved. The low LODs and low RSDs indicate that the technique has great potential and stability for analyzing.

## 6.6 REFERENCES

- [1] H.B. Lee, L.D. Weng, A. Chau, *J. Assoc. Off. Anal. Chem.* 67 (1984) 789.
- [2] F. Navarro-Villoslada, L.V. Perez-Arribas, M.E. Leon-Gonzalez, L.M. Polo-Diez, *Anal. Chim.* 381 (1999) 93.
- [3] F. Hernandez, J. Beltran, F.J. Lopez, J.V. Gaspar, *Anal. Chem.* 72 (2000) 2313.
- [4] V.M. Leon, B. Alvarez, M.A. Cobollo, S. Munoz, I. Valor, *J. Chromatogr. A* 999 (2003) 9.
- [5] Y. Hee, H.K. Lee, *Anal. Chem* 69 (1997) 4643.
- [6] L. Chimuka, E. Cukrowska, J. A. Johnson. *Pure Appl. Chem.* 76 (2004) 707.

- [7] Z. Limian, H.K. Lee, *J. Chromatog. A* 919 (2001) 381.
- [8] M.B. Melwanski, S.-D. Huang, M.-R. Fuh, *Talanta* 72 (2007) 373.
- [9] M.A. Jeannot, F.F. Cantwell, *Anal. Chem.* 68 (1996) 2236.
- [10] M.A. Jeannot, F.F. Cantwell, *Anal. Chem.* 69 (1997) 235.
- [11] V. He, H.K. Lee, *Anal. Chem.* 69 (1997) 4634.
- [12] L. Zhao, H.K. Lee, *J. Chromatogr. A* 919 (1999) 381.
- [13] S. Pederson-Bjergaard, K.E. Rasmussen, *Anal. Chem.* 71 (1999) 2650.
- [14] Stuart, B.H, *Polymer analysis*. England: John Wiley & Sons, LTD. 2002.
- [15] E. Psillakis, N. Kalogerakis. *J. Chromatogr. A* 938 (2001) 117.
- [16] A. Tor, M. Aydin. *Anal. Chim.* 575 (2006) 138.